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LIPASE CATALYSED SYNTHESIS OF SUGAR ESTER IN ORGANIC SOLVENTS

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Summary

The synthesis of sugar esters catalysed by lipase in organic solvents was studied. Immobilized *Candida* and *Mucor miehei* lipase catalysed the synthesis of fructose and glucose esters of stearic acid in tertiary butyl alcohol with yields of 10 to 24 %. In the presence of phenyl or butyl boronic acid synthesis of glucose ester was achieved in hexane, heptane, benzene and toluene. The only positive reaction on disaccharides was found with palatinose.

Introduction

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are widely distributed among animals, plants, and microorganisms. They catalyse the hydrolysis of ester bonds at the fat/water interface. Binding to emulsified triglyceride substrates markedly increases their hydrolytic activity. They are used in the fat and oil and detergent industries. They can also catalyse the synthesis of esters with desirable properties for industrial applications in organic solvents (Klibanov *et al.* 1987, Zaks and Russell. 1988; Deetz & Rozzell 1988; Wong et. al. 1990). Enzyme catalysed regioselective acylation of sugars offers an alternative to the poor selectivity of chemical synthesis (Dordick, 1992). In addition, enantioselective biocatalysis in nonaqeous media is becoming increasingly important in preparative synthetic chemistry (Chen & Sih 1989). Fatty acid esters of carbohydrates constitute an interesting group of nonionic surfactants which are widely used in the pharmaceutical, cosmetic, petroleum and food industries. They are an integral part of many industrial, agricultural and food processes (Seino & Uchiboro 1984; Shaw & Klibanov 1987; Björkling *et. al.* 1989). The objectives of this work were to screen lipases for the synthesis of sugar esters.

Material and Methods

Materials:

The following lipases were used in this study: Lipozyme TM 20 (*Mucor miehei*) and SP 382 (*Candida sp.*) from NOVO Industries, *Humicola lanuginosa* lipase and *Pseudomonas fluorescens* lipase from Biocatalysts Ltd..

The following solvents were used: tertiary butyl alcohol (TBA) from Fluka AG, pyridine, tertiary butyl methyl ether (TBE), dioxan, 2-methyl 2,4-pentadiol, toluene, hexane and heptane from Merck.

The following sugars were studied: anhydrous glucose from Merck, 3 h-vacuum dried glucose and fructose from Sigma, maltose from Riedel de Haen, palatinose was supplied by Prof. K. Bucholz, (Institut für Zuckerindustrie, Braunschweig), sucrose from Serva.

The following fatty acids were investigated: butyric,- caproic,- lauric,- myristic,- palmitic,- oleic,- stearic,- arachidonic acid from Sigma.

Phenylboronic acid, butylboronic acid, chloroform, methanol and acetic acid were purchased from Sigma.

Synthesis of sugar esters:

Experiments were carried out in 5 ml capped tubes with 100 mM of the respective sugar, 100 mM stearic acid, 2 ml tertiary butyl alcohol and 40 mg immobilized enzyme in a heating/stirring module (Pierce, Rockford, Il, USA) at tempertures indicated below. Control experiments were set up in which only the sugar, stearic acid and solvent were incubated. On a preparative scale the reaction was carried out in 500 ml flasks with 50 mM of fructose, 50 mM stearic acid, in 200 ml TBA with 200 mg lipase SP382 in an ofen (Memmert OM 400, Schwabach, Germany) at 40 °C by slow agitation using a magnetic stirror (Ikamac, Staufen, Germany). In the preparative scale the given concentration of the components resulted in highest productivity.

Determination of reaction products:

The progress of the reaction was followed by thin layer chromatography (TLC) developed in a chloroform: methanol: acetic acid: water (70:20:8:2) solvent. For quantitative determination of sugar esters a separation by HPLC was carried out on a Knauer pump with a Knauer 50 gradient former and a Sedex 45 light scattering detector (SEDRE). Separation was achieved on a Nucleosil C18, 5 μm column (250 X 4.6 mm) with a solvent system of methanol/acetic acid (99.7/0.3) at a flow rate of 1.0 ml/min at 20 °C. Quantitative data were obtained from peak areas with a Shimadzu CR 5A integrator.

The amount of water in the solvent and reaction mixture was determined by the Karl Fischer method (Mettler D18 titrator, Mettler, Switzerland).

Results and Discussion

TLC analysis of the reaction of stearic acid with different sugars in tertiary butyl alcohol (TBA) catalysed by lipozyme TM20 showed, glucose ester spots at 24 h, 48 h, and 72 h. With fructose a prominent fructose ester spot was observed at 24 h, 48 h and 72 h but in comparison the glucose ester spots were very light. No sugar ester spots were observed with the other sugars.

A typical HPLC chromatogram is shown in figure 1. The sugar was eluted at 2.4 - 2.8 min followed by the sugar ester at 3.8 - 4.3 min and the stearic acid at 5.20 - 5.75 min.

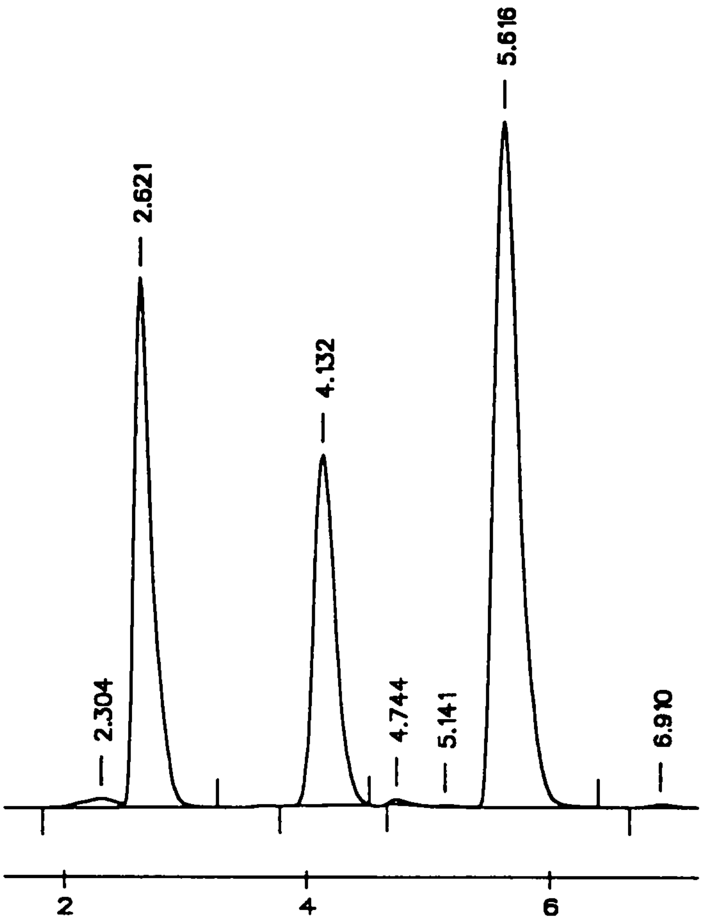

Figure 1. HPLC of a sample incubated with lipase SP 382 at 40 °C for 24 h.

Catalysis with different enzymes in TBA:

Esterification of glucose and fructose with stearic acid in TBA by lipase from *Candida sp., H. lanigunosa and Pseudomonas flourescens* was investigated. After 24 h fructose and glucose ester spots were observed with *Candida sp.* lipase but, no reaction products were observed with lipase from *H. lanigunosa* and *P. fluorescens*.

Reaction in the presence of organicboronic acid:

Organicboronic acids are known to solubilize sugar by forming a carbohydrate-boronate complex by reversible condensation with carbohydrates. The carbohydrate-boronate complex is usually soluble in nonpolar organic solvents and readily hydrolyzed by small amounts of water (Ferrier 1972). Taking advantage of this reaction, synthesis of carbohydrate ester was investigated in different solvents (benzene, 1,4 dioxane, heptane, hexane, pyridine, tertiary butyl ether and toluene) using 20 mg/ml Lipozyme TM 20, 100 mM glucose, 100 mM stearic acid and

100 mM phenylboronic or butyl boronic acid. All solvents were dried on a molecular sieve of 4 Å (0.5 g/10 ml solvent). The initial amount of water in the solvent did not exceed 0.17 % (0.1 - 0.17). Depending on the reaction time and the addives the water content was 0.3 - 0.9 % at the end of the reaction period. Table 1 shows the concentration of sugar ester formed in the presence of activators (phenylboronic acid and butylboronic acid). Phenylboronic acid is a better activator than butylboronic acid. In the presence of an activator heptane is the best solvent, followed by benzene.

Table 1. Effect of organic boronic acid on glucose ester production with Lipozyme TM 20.

Solvent	Concentration of glucose ester formed	
(μmole/ml)	phenylboronic acid	butylboronic acid
benzene	6.75	0.0
heptane	9.32	6.68
hexane	5.44	5.11
tertiary methyl ether	5.67	0.0
toluene	6.06	0.0
dioxane	4.81	0.0
tertiary butyl alcohol*	4.53	n. d.

Reaction period 46 h at 40°C. * boronic acid was not added.

After incubation for 46 h at 40 °C glucose ester formation was observed with benzene, heptane, hexane, tertiary butyl ether and toluene but not with pyridine. This is contrary to the report of Chopineau *et al.* (1988) who used *Chromobacterium viscosum* lipase and Therisod & Klibanov (1986) who used porcine pancreatic lipase. Glucose ester was also formed in dioxane without the addition of boronic acid. The extent of the reaction corresponded to the increasing hydrophobicity of the solvents as has been observed with other enzymes by Zaks, & Klibanov (1985), Klibanov (1989) and Chen & Sih (1989). Lipase from neither *Humicola l.* nor *Pseudomonas spec* catalysed the synthesis of sugar esters even in the presence of either phenylboronic or butylboronic acid. Of all the dissacharides tested only palatinose showed a positive reaction with a yield of 4.93 μmoles/ml.

Figure 2 shows the effect of different fatty acids on fructose ester formation.

The activity tends to increase with the number of carbon atoms in the fatty acid. SpA382 shows a broad specificity similar to that observed in a hydrolysis reaction (Macrae 1983). The time dependent reaction profile for sugar ester production with both, lipase TM 20 and SP 382, is shown in figure 3.

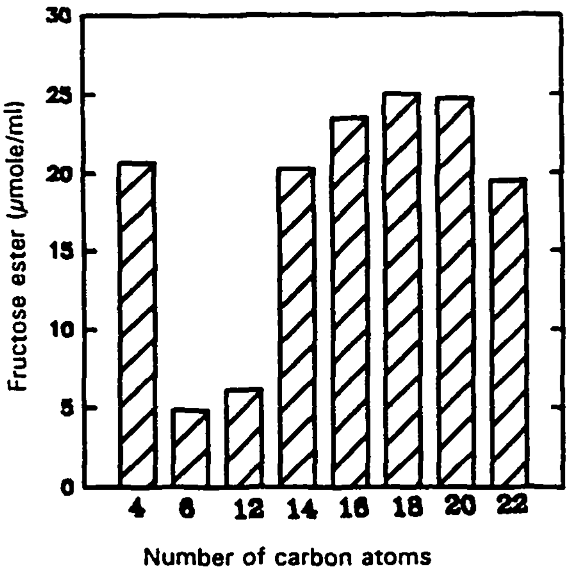

Figure 2. Production of fructose ester by lipase SP 382 with different fatty acids (100 mM) and fructose (100mM) in TBA at 40 °C.

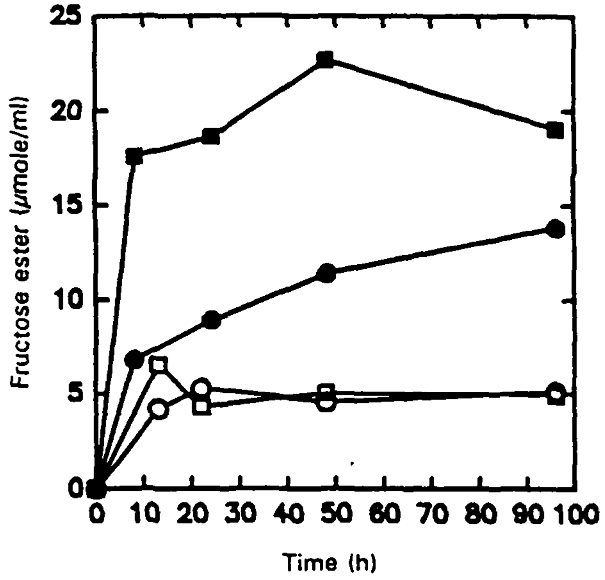

Figure 3. Production of fructose (closed symbols) and glucose (open symbols) ester by lipase TM 20 (circles) and lipase SP 382 (squares) with stearic acid in TBA at 40 °C.

The highest concentration of sugar esters was obtained with SP 382 on fructose and TBA as the solvent. With TM 20 the yield was 8.9% (fructose ester) and 5.3% (glucose ester). With SP 382 the yield was 8.6% (fructose ester) and 4.3% (glucose ester). Fructose ester was purified (HPLC) and identified by NMR. The esterification occurs at position 1 or 6 of the fructose. The determination of the position of the ester bond is under investigation.

A fructose oleate synthesis in a fixed catalyst bed reactor was reported to yield 83 % when the effluent was recycled (Khaled et. al. 1991).

Enzymes have been reported (Zaks & Klibanov 1984) to be active at high temperatures in organic solvents. The effect of temperature on sugar ester formation with the two enzymes was investigated in the temperature range of 40 - 100 °C. Samples where taken hourly up to 8 hours and a final sample was drawn after 24 hours of incubation. Both enzymes exhibited an optimum production at 60 °C with 10 % higher yields of sugar ester then at 40 °C. The activity of TM 20 decreased after 60°C to the initial rate at 40 °C, while SP 382 remained at the optimum level in the range of 60 - 100°C.

Conclusion

Both fructose and glucose esters were formed from stearic acid in TBA with immobilized lipase TM 20 and SP 382. Lipase SP 382 was the best catalyst of all the enzymes investigated, being able to catalyse the reaction without the use of an activator and yielding the highest concentration of product. Glucose ester was formed from stearic acid in 1,4 dioxane with TM 20 as the catalyst. In the presence of butylboronic acid or phenylboronic acid glucose ester was formed from stearic acid in benzene, heptane and hexane, tertiarybutylmethy ether and toluene. The highest productivity of sugar esters was achieved by lipase TM 20 with fructose in the presence of phenylboronic acid.

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